(23)

23RD PLANT DEVELOPMENT WORKSHOP

BIOLOGY DEPARTMENT, MCMASTER UNIVERSITY

SATURDAY, MAY 6TH, 1989

9:00 - 9:30 a.m.	Reception
9:30 - 9:50 a.m.	D.T. Kudirka. Temporal differences in cell cycle activity between tissues of the petal of *Tradescantia*.
9:50 - 10:10 a.m.	Hilary R. Nield and J.N.A. Lott. Microscopy study of *Cucurbita maxima* and *Cucurbita andreana* pollen.
10:10 - 10:30 a.m.	T.C. Charles and T.M. Finan. Analysis of a *Fix* region on the *Rhizobium meliloti* megaplasmid pRmeSU47b.
10:30 - 10:50 a.m.	S.F. Baum, R. Aloni and C.A. Peterson. Role of cytokinins in xylem regeneration in *Coleus blumei* internodes.
10:50 - 11:10 a.m.	Coffee
11:10 - 11:30 a.m.	R. Aloni, S.F. Baum and C.A. Peterson. Role of cytokinins in phloem regeneration in *Coleus blumei* internodes.
11:30 - 11:50 a.m.	B. Moffatt, M. Laloue, C. Pethe and C.R. Somerville. Metabolism of cytokinin bases by adenosine phosphoribosyltransferase in young Arabidopsis plants.
11:50 - 12:10 p.m.	D. Davidson. Plastids: Analysing their role in cellular economy.
12:10 - 12:30 p.m.	T.R. Finan, O.K. Yarosh and T.C. Charles. Analysis of C_4-dicarboxylate transport genes in *Rhizobium meliloti*.
12:30 - 1:50 p.m.	Lunch (Second Floor Lounge - 213)
1:50 - 2:10 p.m.	V.R. Bommineni, D.B. Walden, and R.I. Greyson. Maize (*Z. mays* L.) meristem culture: some feasibility studies.
2:10 - 2:40 p.m.	L. Erickson, E. Swanson and L. McLellan. Haploid tissue systems for translation of *Brassica*.
2:40 - 3:10 p.m.	A.R. Paterson. The Royal Botanic Garden.

Temporal Differences in Cell Cycle Activity between Tissues of the Petal of Tradescantia.

D.T. Kudirka, Department of Plant Sciences, University of Western Ontario, London, Ontario

Tradescantia is a monocot with floral organs arranged as an inflorescence. The spatial arrangement of flower buds in the inflorescence was used to analyze terminal stages of petal growth and development. These stages included a period of mitotic activity which ceased with cells arresting in the G_1 stage of the cell cycle. Following this period of arrest but before pigmentation and anthesis there was a discrete shift of a major proportion of cells from the G_1 to the G_2 stage of the cell cycle. Mitotic frequencies which were analyzed in the i) upper and lower epidermis ii) mesophyll and iii) provascular-vascular tissue showed the timing of cell divisions in the provascular-vascular tissue to be slightly different from the other tissues; however, a cyto photometric survey of cells from the epidermis-mesophyll and provascular-vascular tissues showed the cells of the epidermis-mesophyll to shift earlier in development from the G_1 to the G_2 stage of the cell cycle than the cells of the provascular-vascular tissue.

NIELD, HILARY R. AND JOHN N. A. LOTT. Biology Department, McMaster University, Hamilton, Ontario, L8S 4K1, Canada.
A Light Microscopy Study of Cucurbita maxima and Cucurbita andreana Pollen.

Very little is known about the storage reserves or structure of Cucurbita pollen. Preliminary studies of Cucurbita maxima and Cucurbita andreana pollen structure and storage reserves were carried out using light microscopy. Pollen sections were stained with either a general oversight stain or a histochemical stain. The stains helped to characterize the pollen cytoplasm and to localize storage reserves. The results showed that both Cucurbita maxima and Cucurbita andreana pollen contained similar storage reserves. The histochemical stains confirmed that the primary storage product of Cucurbita pollen appears to be starch. Other reserves are composed of lipids and proteins. Light microscopy studies of Cucurbita pollen serve as a valuable supplement to fine structural studies of pollen.

Analysis of a Fix Region on the Rhizobium meliloti Megaplasmid pRmeSU47b.

Trevor C. Charles and Turlough M. Finan.
McMaster University.

The >1500 kb megaplasmid pRmeSU47b is one of two megaplasmids in the alfalfa symbiont Rhizobium meliloti. Both of these megaplasmids carry genes involved in the formation of nitrogen-fixing root nodules on alfalfa. We have constructed a genetic linkage map of pRmeSU47b, and have generated large defined deletions of regions flanked by transposon insertions.

By analysis of these deletions we have identified a new locus on pRmeSU47b which is required for symbiotic N2 fixation (Fix-). Clones which restore the Fix- deletion mutants to Fix+ were isolated from a cosmid bank of wild type R. meliloti DNA. A 7.3 kb BamH1 fragment, subcloned into the IncP plasmid pRK7813, was shown to restore the deletion mutants to Fix+. The region has been further delineated by site-directed Tn5 mutagenesis, and gene organization and expression are being investigated using translational fusions to the E. coli reporter gene phoA (ie. alkaline phosphatase).

BAUM, STUART F., RONI ALONI* and CAROL A. PETERSON. Department of Botany, Tel Aviv University, Tel Aviv 69978 and Department of Biology, University of Waterloo, Waterloo, Ontario, N2L 3G1 - Role of cytokinins in xylem regeneration in Coleus blumei internodes.

The effects of three cytokinins in the regeneration of vessels around a wound were studied in mature internodes of Coleus blumei. Zeatin, kinetin or BAP (6-benzylamino-purine) were applied in aqueous solutions to the bases of the excised internodes. Auxin (IAA) was applied to the apical ends of all the internodes. The controls with no cytokinin exhibited a low level of vascular regeneration around the wound. At appropriate concentrations, all three cytokinins induced a significant increase in vessel regeneration. Additional patterns of supplementary vessel differentiation were observed at some distance from the wound. The regeneration of vessels induced by cytokinins was very polar. Many more regenerated vessel members differentiated below the wound than above it, and the regenerative process proceeded acropetally from the base of the internode to its upper parts.

ALONI', RONI, STUART F. BAUM, and CAROL A. PETERSON Department of Botany, Tel Aviv University, Tel Aviv 69978 and Department of Biology, University of Waterloo, Waterloo, Ontario, N2L 3G1 - Role of cytokinins in phloem regeneration in Coleus blumei internodes.

The effects of cytokinin on sieve tube regeneration, callose levels and the relationship between phloem and xylem regeneration were studied in wounded internodes of Coleus blumei. Cytokinin was applied in aqueous solution to the bases of wounded internodes; auxin was applied to their apical ends. Optimal cytokinin concentrations induced more regenerated sieve tubes than endogenous concentrations in the controls. Kinetin was most effective at 5 or 10 mg/L whereas zeatin showed the greatest effect at 20 mg/L. Under a low auxin level (0.1 %) zeatin was more effective, whereas kinetin was the effective cytokinin under a high (1 %) IAA concentration. Cytokinin induced high amounts of callose on the sieve plates, especially at the highest concentration (50 mg/L), facilitating observation of the regenerated sieve tubes. In all the cytokinin treatments as well as in the control there were always more regenerated sieve tubes than regenerated vessels.

METABOLISM OF CYTOKININ BASES BY ADENINE PHOSPHORIBOSYL TRANSFERASE IN YOUNG ARABIDOPSIS PLANTS

[1]Barbara Moffatt, [2]Michel Laloue, [2]Claude Pethe and [3]Chris Somerville.
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While cytokinins have been shown to exist in a variety of forms, little is known about the mechanisms of their interconversion or the enzymes which effect these changes. In vitro experiments have shown that several purine salvage enzymes can act on cytokinin bases and ribosides. We have taken a genetic approach to determine whether one purine salvage enzyme, adenine phosphoribosyl transferase (APRT), metabolizes cytokinin bases in addition to adenine, in vivo. A mutant of A. thaliana designated BM3, which is deficient in APRT activity has recently been isolated and characterized. This mutant has about 1% of the APRT activity found in wild type (wt) plants as assayed in crude leaf extracts in vitro or as measured by in vivo feeding experiments with ^{3}H-adenine. Similar assays following the metabolism of ^{3}H-bz^{6}Ade and bz^{6}Ado in both wt and BM3 plants indicate that mutant plants metabolize the riboside normally but do not convert bz^{6}Ade to bz^{6}AMP to a significant extent. Instead, APRT-deficient plants accumulate more of the 7- and 9-glucosides of bz^{6}Ade than wt plants. These results and those of IEF analysis of enzyme activity indicate that APRT is the main enzyme which converts bz^{6}adenine to bz^{6}AMP in young Arabidopsis plants. Further, these results demonstrate that the conversion of bz^{6}Ade to a nucleotide form is not related to its uptake.

PLASTIDS: ANALYSING THEIR ROLE IN CELLULAR ECONOMY. D. Davidson, Biology Department, McMaster University, Hamilton.

Plastids are important organelles. They are the site of several synthetic events that occur only in plastids; i.e. nowhere else in cells. In part this reflects compartmentalization of enzymes encoded by nuclear genes; in part it occurs because some enzymes are encoded by plastid genomes (ctDNA). Mutations of ctDNA genes are of special interest because of the special functions controlled by ctDNA. To identify and use ctDNA mutations mutant homoplasmons must be formed: a) to identify the mutant phenotype; b) to use a mutant homoplasmon to generate a whole plant. One factor that delays mutant homoplasmon formation is the multiplicity of copies of ctDNA. When seeds are treated with nitromethylurea, however, mutant homoplasmons form rapidly in several species; e.g. tobacco, corn, cabbage. We suggest that this rapid formation of mutant homoplasmons occurs because seed shoot apical cells contain few ctDNA's. Evidence for rapid formation of mutant homoplasmons is found from: a) % of leaves with mutants sectors; b) % of leaf area occupied by a mutant sector. Data will be presented, for both parameters, that they increase when mutagen dose is increased; they suggest that a mutagen induced inhibition of ctDNA replication temporarily reduces ctDNA molecule number and so facilitates mutant homoplasmon formation.

Analysis of C4-Dicarboxylate Transport Genes in Rhizobium meliloti

Turlough M. Finan, Oksana K. Yarosh and Trevor C. Charles, Department of Biology, McMaster University, 1280 Main Street West, Hamilton, Ontario L8S 4K1, Canada.

In leguminous root nodules the plant supplies the N2-fixing bacteria (bacteroids) with energy in the form of reduced carbon. C4-dicarboxylic acids appear to be the major carbon source utilized by bacteroids and thus it is of importance to provide a molecular characterization of the C4-dicarboxylic acid transport (Dct) system of rhizobia. Characterization of the dct genes of Rhizobium meliloti revealed three loci, designated dctA, dctB, and dctD. Alkaline phosphatase (phoA) gene fusions to dctA suggested that this gene encodes the structural transport protein. Analysis of the fusions in various mutant backgrounds demonstrated that dctB, dctD, and ntrA products are required for dctA expression. Alfalfa plants inoculated with the dctB and dctD mutants showed reduced N2-fixing activity. Nodules induced by dctA mutants failed to fix N2. These symbiotic phenotypes are consistent with previous suggestions that dctA expression in bacteroids can occur independently of dctB, and dctD.

Maize (*Zea mays L.*) meristem culture: some feasible studies

V. R. Bommineni, D. B. Walden, and R. I. Greyson. Department of Plant Sciences, University of Western Ontario, London, ONT.

In vitro culture of the different apical and axillary meristems of maize represent a variety of opportunities for basic and applied research. These include:

1) Shoot apex of the early embryo
2) Shoot apex of the mature embryo
3) Vegetative axillary buds
4) Tassel primordia
5) Ear primordia

A summary of these explants and their potential applications in plant biotechnology will be discussed.

HAPLOID TISSUE SYSTEMS FOR TRANSFORMATION OF BRASSICA

L. Erickson, E. Swanson and L. McLellan, Biotechnology Section, Allelix Crop Technologies, Mississauga, Ontario.

The microspore system in B. napus provides large numbers of relatively synchronous, haploid, single cells with high regeneration capacity. The main advantage of transforming haploid tissue is the immediate fixation of the trait upon regeneration and chromosome doubling with colchicine. This fixation results in greatly simplified genetic analysis and allows rapid transfer of the transferred gene into breeding lines. We are employing both direct DNA transfer methods (electroporation, particle bombardment) and cocultivation with Agrobacterium tumefaciens to transform microspores and microspore-derived embryos at various stages of development. The effects of varying cocultivation parameters are monitored by GUS assays up to about day 10 after microspore isolation after which stage blue embryos begin to a appear in the controls. NPT-II assays are generally ineffective for detecting transformants carrying the nos promoter whereas the development of microspores in medium with kanamycin reliably indicated the presence of the gene, confirmed by Southern analysis.

The Royal Botanic Garden. Dr. A. Paterson, Director of The Royal Botanic Garden, Burlington, Ontario.

Botanic Gardens are generally seen to have a responsibility in the conservation of endangered plant species. Typically, a garden relates this to the flora of its own area; sometimes, as in the case of Missouri Botanic Garden's activities in tropical rain forests of South America, the activity is abroad.

RBG Hamilton's particular concerns are horticultural and conservation thrusts relfect this in the setting up of the Centre for Canadian Horticultural Historical Studies and the recent initiation of a restoration project at the famous garden of Monserrate in Portugal. The plant collection and its future will be discussed in this paper.